



Institute for Tumor Biology

Klaus Pantel, MD, PhD

The potential of circulating tumour cells as a liquid biopsy to guide therapy in prostate cancer



Disclosure Information

Klaus Pantel

I have the following financial relationships to disclose:

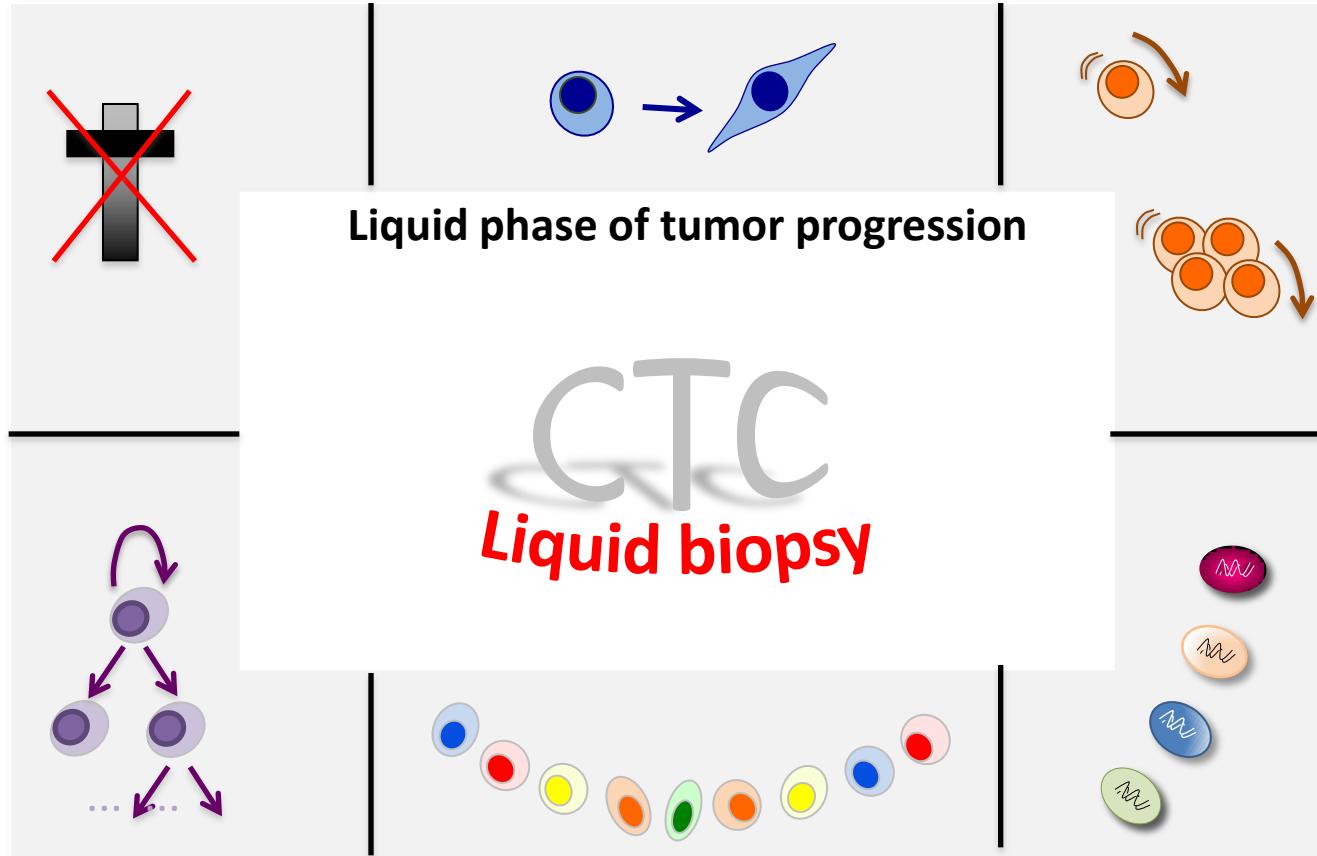
Grant/Research Support from: Veridex/Janssen

Advisory Board: Veridex/Janssen, Alere, Gilipi

Anoikis resistance

Epithelial-to-mesenchymal transition

Invasion/Intravasation ability
(single CTCs and/or clusters)



**The technical challenge:
Finding one tumor cell in 10^6 – 10^8 normal blood cells**

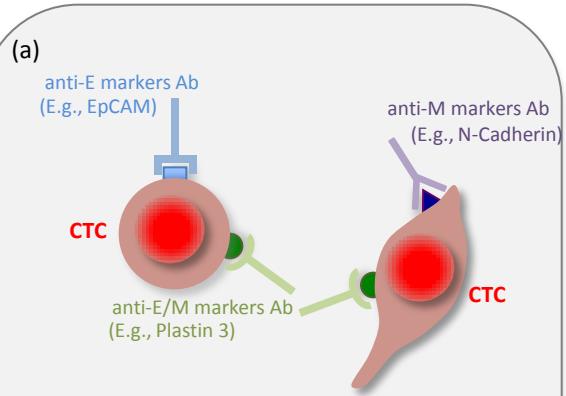
Biological properties

Protein expression

Physical properties

Label-free strategies

Positive Selection



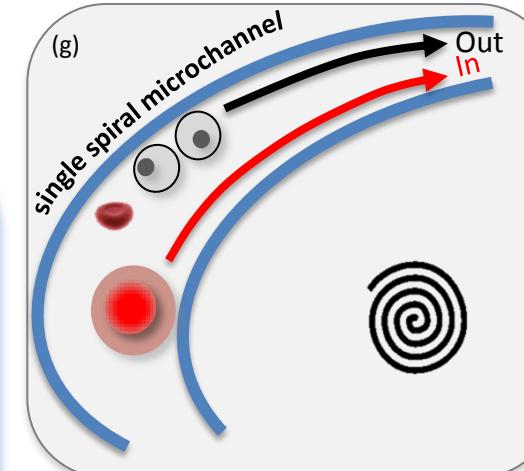
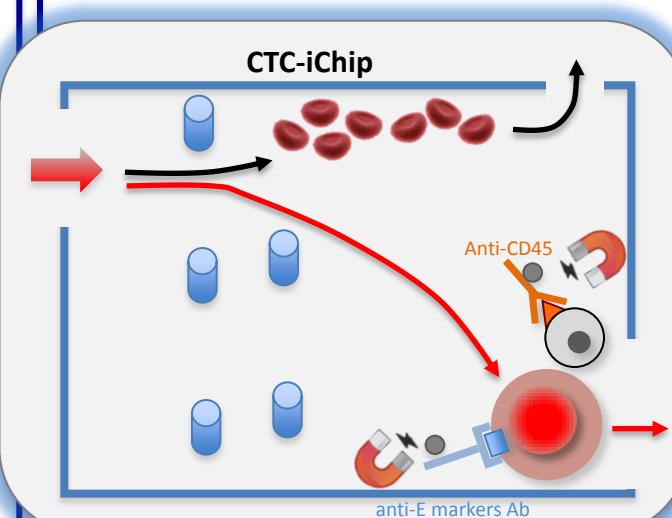
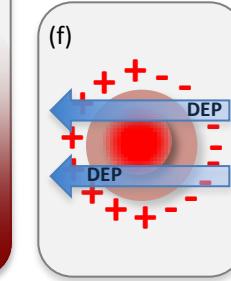
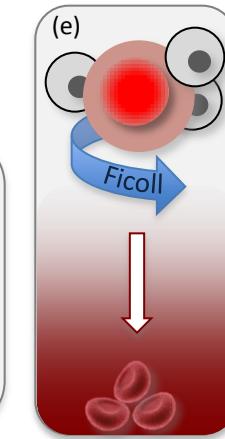
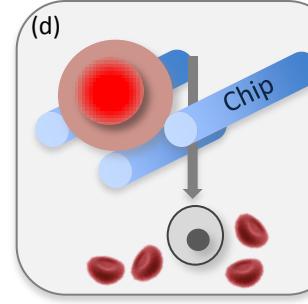
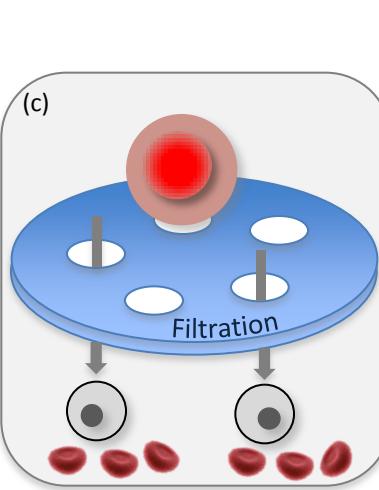
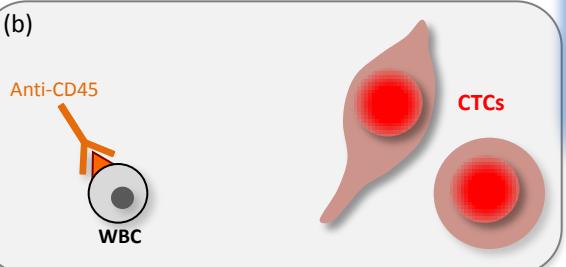
Ex vivo

- CellSearch® system
- MagSweeper™
- EPHESIA CTC-chip
- CTC-chip
- Velcro-like device

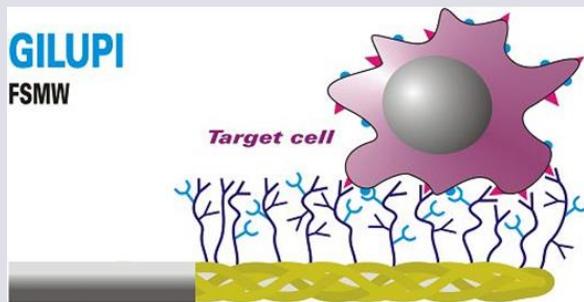
In vivo

- CellCollector®
- Photoacoustic nanodetector

Negative Selection

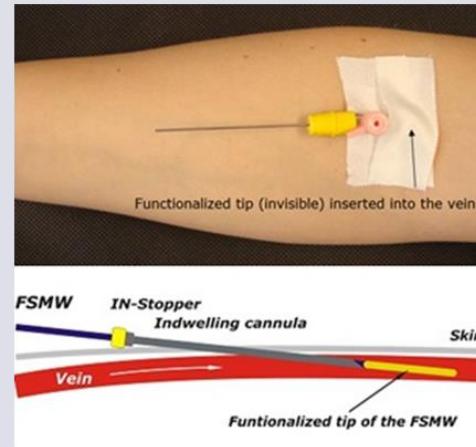


CellCollector™ : *In vivo* capture of CTC



Nanodetector

Insertion into patient's vein at the doctor's office
30 minutes exposure time in a vein



Decision



Result

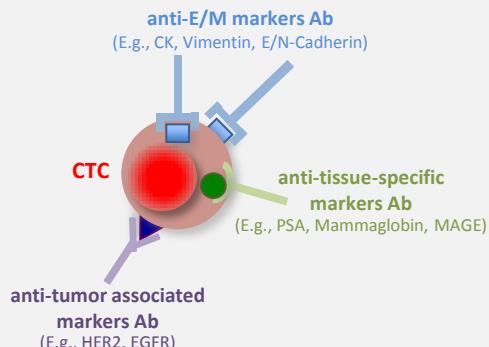


Diagnostics
➤ cytology
➤ PCR, etc.

Proof of principle data in breast, prostate, colon and lung cancer

Approaches for CTC detection

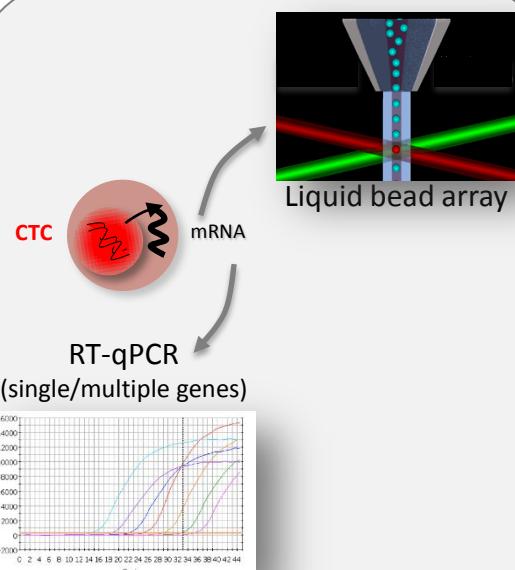
Immunocytological technologies



Technologies

- Immunocytochemistry
- CellSearch® system
- Flow Cytometry
- DEParray®

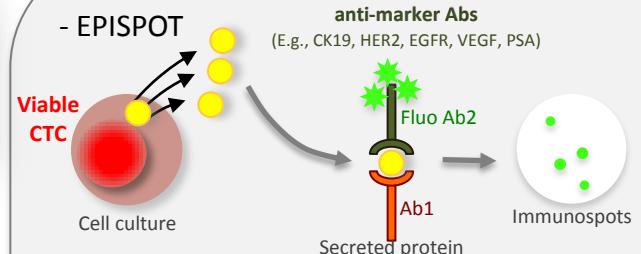
Molecular technologies



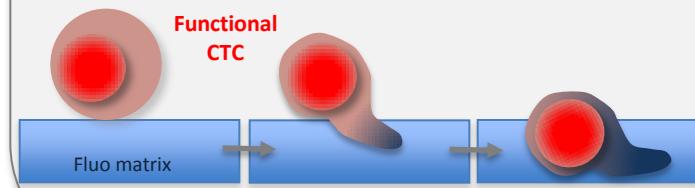
RNA-based Technologies

Functional assays

In vitro Cell Culture



- Invasion assay



Xenotransplantation models (CDx)



Alix-Panabieres & Pantel, *Nature Rev. Cancer* 2014

CellSearch™ System (FDA-cleared)



7.5ml



MagNest™



Enrichment of CTC with
anti-EpCAM ferro fluids

Cristofanilli et al., NEJM, 2004

Riethdorf et al., CCR, 2007 & 2010

DeBono et al, CCR, 2008

Cohen et al, JCO, 2008

Krebs et al, JCO, 2012



CellTracks® Analyzer II
w/ Linux operating system

Prognostic value of CTC counts for survival in cancer patients with advanced disease

Published Ahead of Print on March 10, 2014 as 10.1200/JCO.2013.51.7417
The latest version is at <http://jco.ascopubs.org/cgi/doi/10.1200/JCO.2013.51.7417>

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Circulating Tumor Cell Counts Are Prognostic of Overall Survival in SWOG S0421: A Phase III Trial of Docetaxel With or Without Atrasentan for Metastatic Castration-Resistant Prostate Cancer

Amir Goldkorn, Benjamin Ely, David I. Quinn, Catherine M. Tangen, Louis M. Fink, Tong Xu, Przemyslaw Twardowski, Peter J. Van Veldhuizen, Neeraj Agarwal, Michael A. Carducci, J. Paul Monk III, Ram H. Datar, Mark Garzotto, Philip C. Mack, Primo Lara Jr, Celestia S. Higano, Maha Hussain, Ian Murchie Thompson Jr, Richard J. Cote, and Nicholas J. Vogelzang

Challenge of CTC detection: Epithelial-Mesenchymal Transition (EMT) of carcinoma cells



Cancer Cell
Previews

Tumor Dissemination: An EMT Affair

Jean Paul Thiery^{1,2,5,*} and Chwee Teck Lim^{3,4}

¹Department of Biochemistry

²Cancer Science Institute

³Department of Bioengineering

⁴Mechanobiology Institute

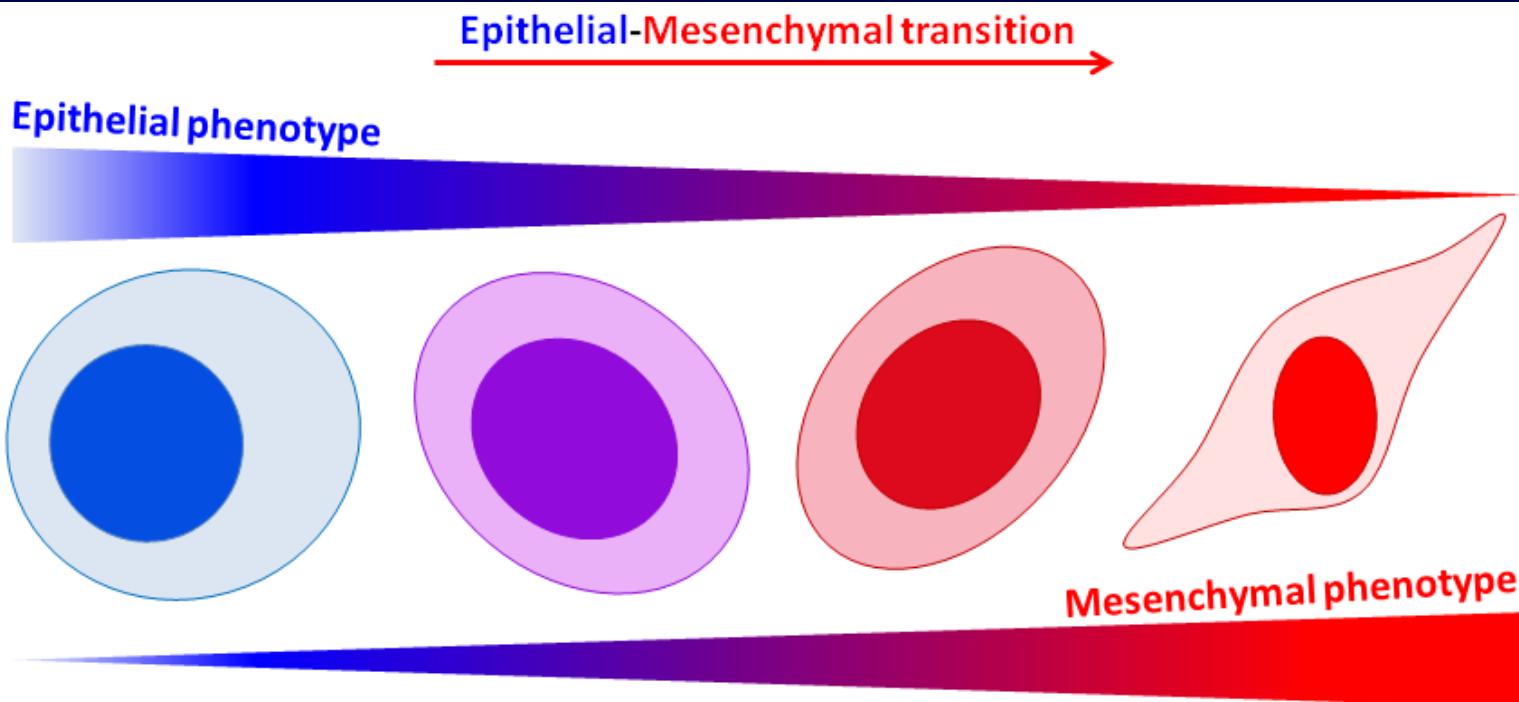
National University of Singapore, Singapore 117599

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<http://dx.doi.org/10.1016/j.ccr.2013.03.004>

Epithelial-Mesenchymal Plasticity of CTC



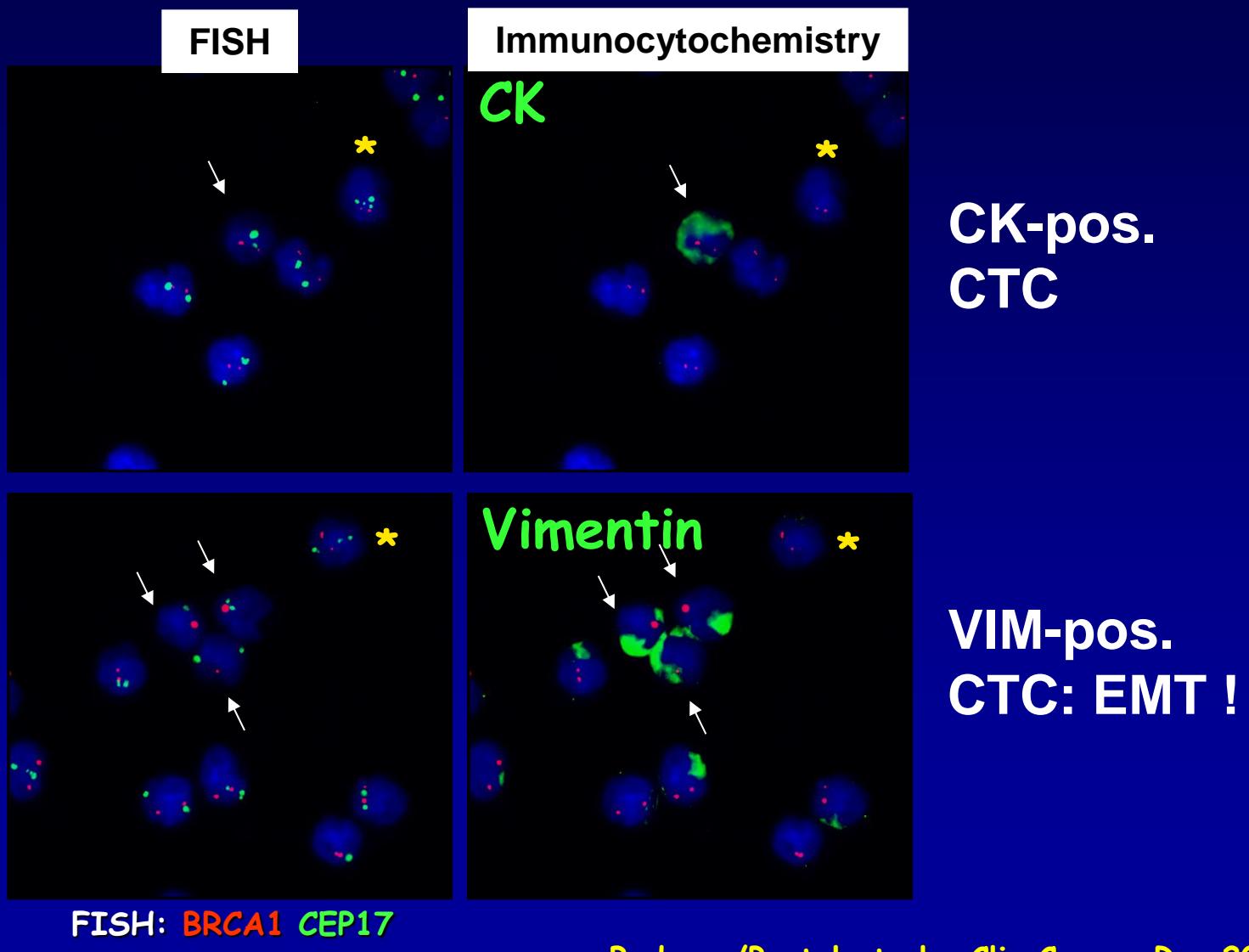
EpCAM, CK

Mesenchymal-Epithelial transition

Vimentin

Epithelial phenotype	Epithelial phenotype with minor mesenchymal features	Semi-mesenchymal phenotype	Mesenchymal phenotype
Epithelial markers strongly expressed	Epithelial markers moderately expressed	Epithelial markers weakly expressed	No epithelial markers
No mesenchymal markers	Mesenchymal markers weakly expressed	Mesenchymal markers moderately expressed	Mesenchymal markers strongly expressed
Detection by standard CTC technology	Detection by standard CTC technology	Limited detection by standard CTC technology	No detection by standard CTC technology

EMT in prostate cancer: BRCA1 gene loss in vimentin-positive CTC



**CTCs as intermediate
endpoint for survival in
clinical trials**

Circulating Tumor Cells (CTC) As An Endpoint in the Abiraterone Phase III trial (mCRPC patients)

Planned Patients

- 1195 patients with progressive mCRPC
- Failed 1 or 2 chemotherapy regimens

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2:1

Abiraterone 1000 mg daily
Prednisone 5 mg BID
n = 797

Placebo daily
Prednisone 5 mg BID
n = 398

Efficacy end points (ITT)

Primary end point:

- OS (25% improvement; HR 0.8)

Secondary/tertiary end points:

TPP, rPFS, PSA response

- **CTC enumeration**

Conversion Rates From Unfavorable (≥ 5 CTC) to Favorable (< 5 CTC) Were Significantly Higher With Abiraterone Acetate Relative to Placebo

	Week 4		Week 8		Week 12	
No. of patients with baseline CTC ≥ 5 and a postbaseline CTC value	422		374		330	
Conversion status	AA (n = 272)	Placebo (n = 150)	AA (n = 245)	Placebo (n = 129)	AA (n = 217)	Placebo (n = 113)
Conversion (n)	42% (113)	14% (21)	50% (123)	17% (22)	48% (105)	17% (19)
P value	< 0.0001		< 0.0001		< 0.0001	

CTCs as Surrogate Marker for Survival

Baseline CTC ≥ 5		
	Week 12 (n = 321, CPE = 0.71 [SE = 0.014])	p Value
Model Factors	HR (95% CI)	
Treatment	1.030 (0.773, 1.372)	0.8371
LDH_FC	1.247 (1.048, 1.483)	0.0127
LDH_BL	3.044 (2.282, 4.056)	<0.0001
CTC Conversion	0.390 (0.289, 0.527)	<0.0001
CTC_BL	1.143 (0.988, 1.323)	0.0729

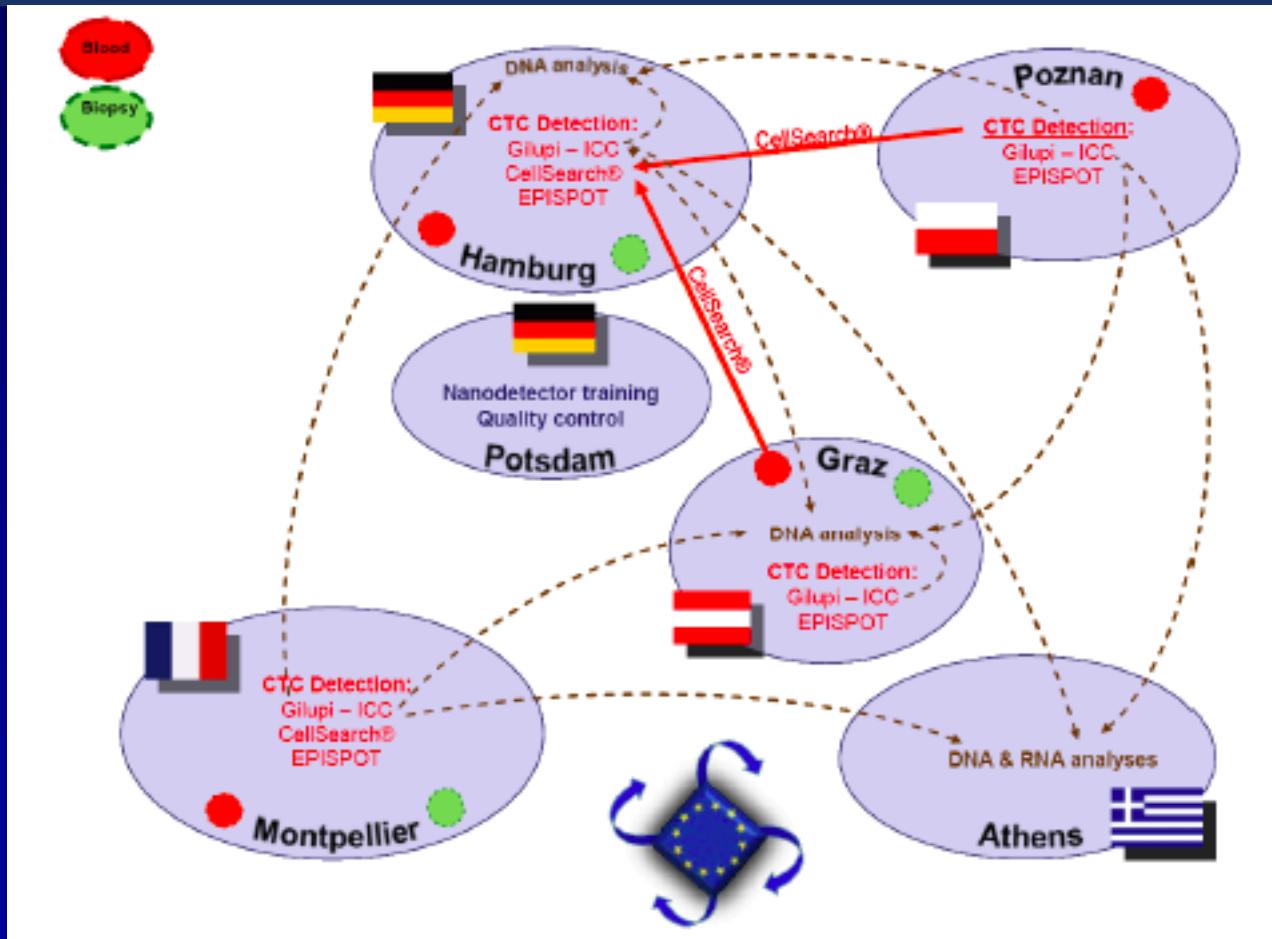
Landmark analysis: CTC conversion: Baseline ≥ 5 and post-baseline < 5
BL, Baseline: FC, Fold change defined as post baseline/baseline value.

CTCs in non-metastatic disease

ERA-NET TRANSCAN: CTC-SCAN Project

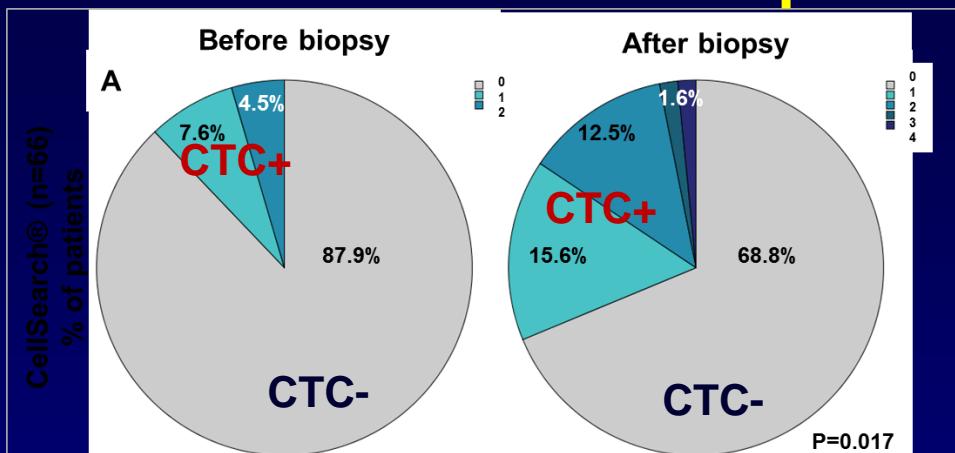
High-risk Prostate Cancer (stage M₀): Prognostic value of CTCs

Partners: Germany, France, Greece, Poland, Austria

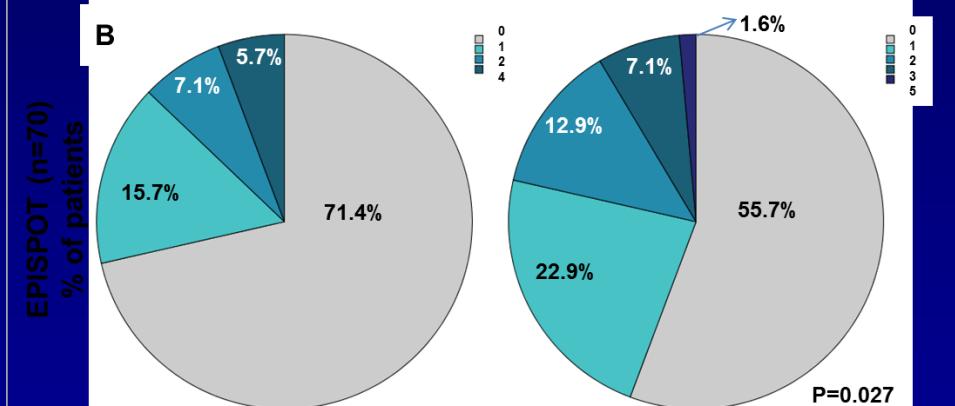


Distribution of CTC-positive cases among peripheral blood samples collected before and after performing biopsy

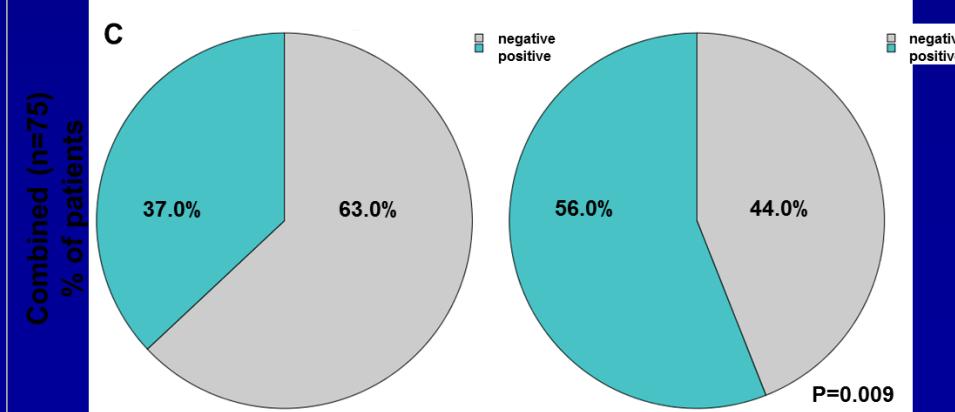
CellSearch
Assay



EpiSpot
Assay



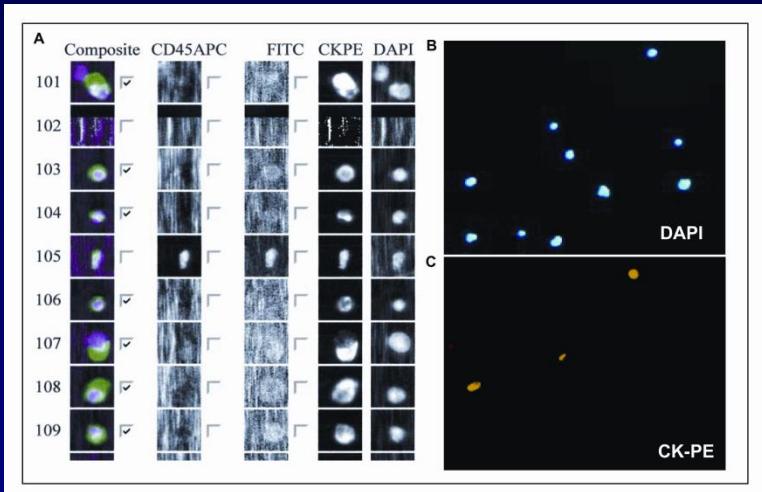
Combined
Results



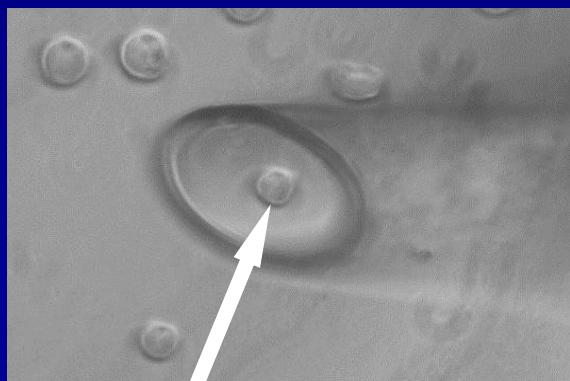
P=0.009

Genomic Characterization of single CTC

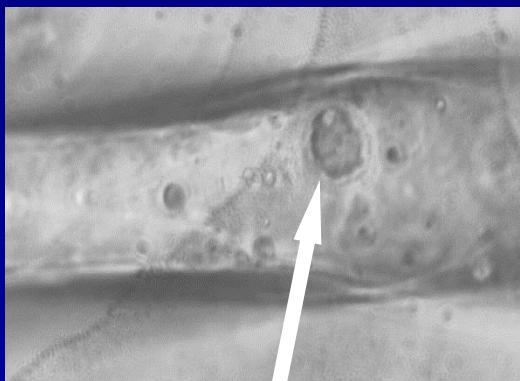
CTC detection



CTC isolation



CTC

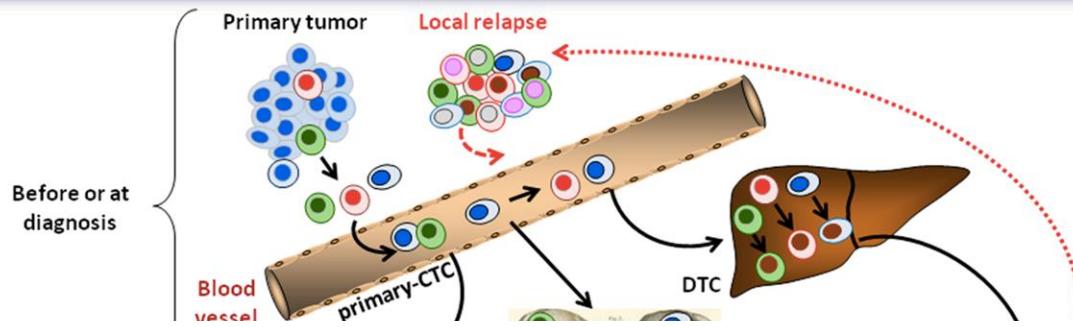


Capillary

CTC

WGA +

- Mutation analysis
- CGH (conv./array)
- NextGen Sequencing



**nature
biotechnology**

Micrometastatic disease

Genotype

ved.

Whole-exome sequencing of circulating tumor cells provides a window into metastatic prostate cancer

Jens G Lohr^{1-3,11}, Viktor A Adalsteinsson^{1,4,11}, Kristian Cibulskis^{1,11}, Atish D Choudhury¹⁻³, Mara Rosenberg¹, Peter Cruz-Gordillo¹, Joshua M Francis^{1,2}, Cheng-Zhong Zhang^{1,2}, Alex K Shalek⁵, Rahul Satija¹, John J Trombetta¹, Diana Lu¹, Naren Tallapragada⁴, Narmin Tahirova⁴, Sora Kim¹, Brendan Blumenstiel¹, Carrie Sougnez¹, Alarice Lowe⁶, Bang Wong¹, Daniel Auclair¹, Eliezer M Van Allen¹⁻³, Mari Nakabayashi^{2,3}, Rosina T Lis², Gwo-Shu M Lee², Tiantian Li², Matthew S Chabot², Amy Ly⁷, Mary-Ellen Taplin^{2,3}, Thomas E Clancy^{2,3,6}, Massimo Loda^{1-3,6}, Aviv Regev^{1,8,9}, Matthew Meyerson¹⁻³, William C Hahn^{1-3,6}, Philip W Kantoff^{2,3}, Todd R Golub^{1-3,9}, Gad Getz^{1,7}, Jesse S Boehm¹ & J Christopher Love^{1,4,10}

CTCs	Treatments
PROTEINS	
ER+	Endocrine therapy
Her2/neu+	Trastuzumab
DNA MUTATIONS	
KRAS mutations	EGFR targeted therapies
PI3K mutations	HER2/neu targeted therapies

CTC as Liquid Biopsy for metastatic cells

Metastasis evolve many years
resection
the genomic

LETTERS

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Information
on metastatic cells located at
different sites ?

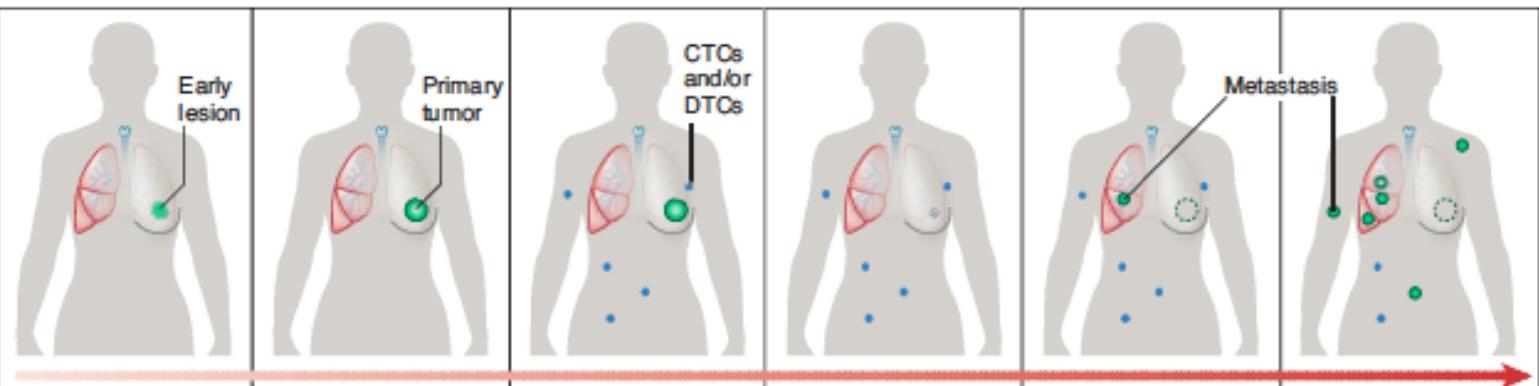
Alix-Panabières & Pantel, *Clin Chem*, 2013; Pantel & Alix-Panabieres, *Cancer Res*. 2013

Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay

Irène Baccelli, Andreas Schneeweiss, Sabine Riethdorf, Albrecht Stenzinger, Anja Schillert, Vanessa Vogel, Corinna Klein, Massimo Saini, Tobias Bäuerle, Markus Wallwiener, Tim Holland-Letz, Thomas Höfner, Martin Sprick, Martina Scharpf, Frederik Marmé, Hans Peter Sinn, Klaus Pantel, Wilko Weichert & Andreas Trumpp



**Metastasis-initiating cells:
EPCAM^{low}, CD44⁺, CD47⁺ and cMET⁺**



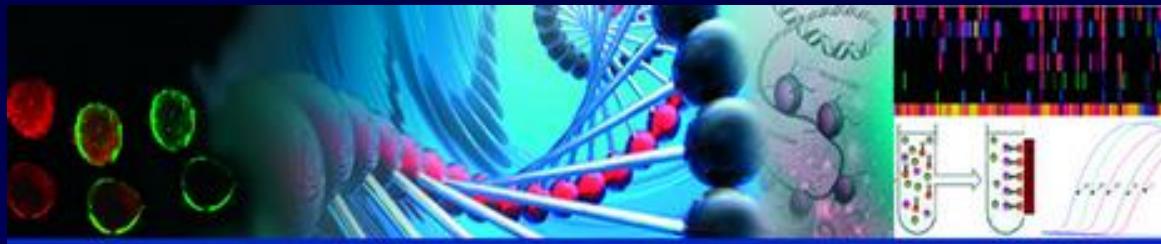
Status	Pre-neoplasm Subclinical	Primary (-) CTCs and/or DTCs	Primary (+) CTCs and/or DTCs	Dormancy	Oligometastases	Systemic metastases		
Focus	Management of primary tumor		Prevention of metastasis		Treatment of metastasis			
Challenge	Early detection and prevention Identify high-risk patients		Prevent local and distant relapse Drug resistance of DTCs		Early detection of relapse Heterogeneity and drug resistance			
New tools	Diagnostic markers	Prognostic markers	Profiling of primary tumor, metastases, CTCs and/or DTCs for accurate targeting Biomarkers and imaging technologies for disease monitoring Biomarkers for therapeutic efficacy					
Possible treatment strategies	Prophylactic treatment Vaccination	Surgery, radiotherapy (+) Systemic therapy		Targeted therapy against driver oncogenes and their pathways tailored by genetic makeup of tumor cells				
			Long-term adjuvant treatment (for high-risk patients): <ul style="list-style-type: none"> Metronomic chemotherapy and anti-angiogenesis Targeting common driver oncogenes and pathways Immunotherapy Targeting dormancy-related survival and CSC signaling and niche components 			Systemic therapy Immunotherapy Stroma-targeting treatments Palliative radiation and/or surgery		
				Surgery stereotactic radiotherapy				
Possible new targets		DTC and/or CTC survival pathways; stem cell features; tumor-stroma crosstalk and niche factors Activation of metastasis-suppressive signaling						



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 - Simon Joosse, Tobias Gorges
 - Kai Bartkowiak, Natalia Bednarz-Koll
- Klaus Pantel, Hamburg:**
- ERC Advanced Investigator Grant**
- „DISSECT“ (2011-2016)**
- Roggenbuck-Stiftung

Micrometastasis Research Network at UCCH/UKE





www.actc2014.org

Advances in Circulating Tumour Cells (ACTC): from Basic Research to Clinical Practice



Save the Date

October 8th - 11th, 2014
Crete, Greece

Organizers:

- Evi S. Lianidou, Department of Chemistry, University of Athens, Greece
- Dimitris Mavroudis, School of Medicine, University of Crete - Department of Medical Oncology, University General Hospital of Heraklion, Greece
- Klaus Pantel, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany
- Hellenic Oncology Research Group

Organizers:
Evi Lianidou, Athens
Dimitris Mavroudis, Crete
Klaus Pantel, Hamburg



**SAVE THE DATE
ISMRC
2015**

10th International Symposium on Minimal Residual Cancer

**September, 2015
Hamburg, Germany**



Klaus Pantel, MD, PhD

See you in 2015 in Hamburg, Germany!